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ISSUE DATE: *April 05, 2011*

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C. Hightower

C. HIGHTOWER
Certifying Officer

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(12) **United States Patent**
Boschelli et al.

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(54) **4-ANILINO-3-QUINOLINECARBONITRILES FOR THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML)**

(75) Inventors: **Frank Boschelli**, New City, NY (US); **Jennifer M. Golas**, West Milford, NJ (US); **Kim T. Arndt**, Towaco, NJ (US)

(73) Assignee: **Wyeth LLC**, Madison, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 403 days.

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Related U.S. Application Data

(63) Continuation of application No. 10/980,097, filed on Nov. 3, 2004, now Pat. No. 7,417,148.

(60) Provisional application No. 60/517,819, filed on Nov. 6, 2003.

(51) **Int. Cl.**
C07D 215/38 (2006.01)

(52) **U.S. Cl.** **546/159**; 546/157; 514/313

(58) **Field of Classification Search** 546/159, 546/157; 514/313

See application file for complete search history.

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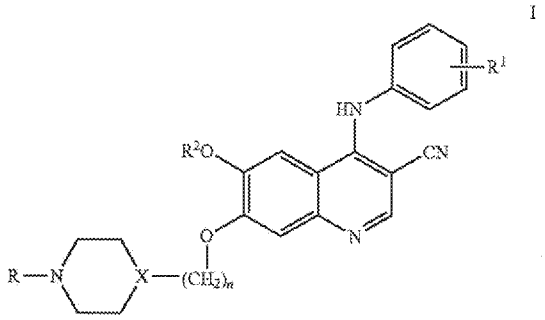
Primary Examiner — D. Margaret Seaman
(74) *Attorney, Agent, or Firm* — David Rubin; Jeffrey H. Tidwell

(57) **ABSTRACT**

Compounds of the formula:



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wherein:
n is an integer from 1-3;
X is N, CH, provided that when X is N, n is 2 or 3;
R is alkyl of 1 to 3 carbon atoms;
R^1 is 2,4-diCl, 5-OMe; 2,4-diCl; 3,4,5-tri-OMe; 2-Cl, 5-OMe; 2-Me, 5-OMe; 2,4-di-Me; 2,4-diMe-5-OMe, 2,4-diCl, 5-OEt;
R^2 is alkyl of 1 to 2 carbon atoms, and pharmaceutically acceptable salts thereof.

1 Claim, No Drawings

4-ANILINO-3-QUINOLINECARBONITRILES FOR THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML)

This application is a continuation application of copending application, application Ser. No. 10/980,097 filed Nov. 3, 2004 which claims priority from provisional application, Application No. 60/517,819 filed on Nov. 6, 2003. These applications are herein incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

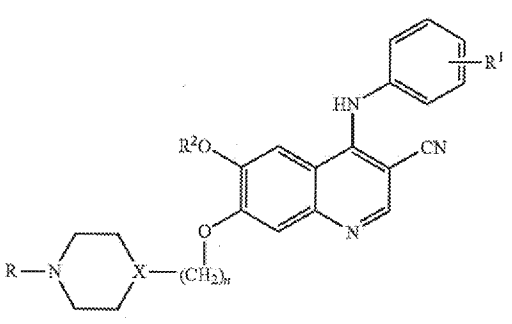
Constitutive tyrosine kinase activity of Bcr-Abl promotes proliferation and survival of chronic myelogenous leukemia (CML) cells. Inhibition of Bcr-Abl tyrosine kinase activity or signaling proteins activated by Bcr-Abl in CML cells blocks proliferation and causes apoptotic cell death. The selective Abl kinase inhibitor, STI-571 (marketed as Gleevec), is toxic to CML cells in culture, causes regression of CML tumors in nude mice, and is currently used to treat CML patients.

Expression of Bcr-Abl in hematopoietic stem cells promotes transformation and acts early in leukemogenesis. Inhibition of this kinase with STI-571 effectively controls CML in the chronic phase of the disease but more advanced patients frequently progress on STI-571 therapy. These observations suggest that additional molecular changes that are not affected by STI-571 play a role in advanced disease. In vitro models of STI-571 resistance and clinical specimens from resistant patients demonstrated that overexpression of other kinases or activation of distinct signaling pathways is associated with Bcr-Abl independence. Inhibition of the tyrosine kinase activity of Bcr-Abl is an effective strategy for targeting CML as demonstrated by the clinical efficacy of STI-571. Other molecules, including Src family kinases, play a role in downstream signaling from Bcr-Abl, and as such, are potential therapeutic targets for the treatment of STI-571-resistant disease. Src family kinases including Lyn and Hck have been implicated in downstream signaling from Bcr-Abl.

Although the selective Abl kinase inhibitor STI-571 is efficacious and well tolerated by most patients in chronic-stage CML, patients in accelerated and blast crises stages of the disease tend to be less responsive. Consequently, there is a need for alternative agents that are effective in late-stage disease.

BRIEF SUMMARY OF THE INVENTION

In accordance with the present invention are provided compounds of the structural formula I:



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wherein:

n is an integer from 1-3;

X is N, CH, provided that when X is N, n is 2 or 3;

R is alkyl of 1 to 3 carbon atoms;

R^1 is 2,4-diCl, 5-OMe; 2,4-diCl; 3,4,5-tri-OMe; 2-Cl, 5-OMe; 2-Me, 5-OMe; 2,4-di-Me; 2,4-diMe-5-OMe, 2,4-diCl, 5-OEt;

R^2 is alkyl of 1 to 2 carbon atoms, and pharmaceutically acceptable salts thereof.

The compounds of this invention may be used for treating, preventing, or inhibiting CML. In a preferred embodiment the compounds are used as part of a pharmaceutical composition.

Specific compounds of the invention include:

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[3-(4-ethyl-1-piperazinyl)propoxy]-6-methoxy-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[2-(4-ethyl-1-piperazinyl)ethoxy]-6-methoxy-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[2-(1-methylpiperidin-4-yl)ethoxy]-3-quinolinecarbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(1-methylpiperidin-4-yl)propoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[(1-ethylpiperidin-4-yl)methoxy]-6-methoxyquinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(4-ethylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(1-methyl piperidin-4-yl)propoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[2-(1-methylpiperidin-4-yl)ethoxy]quinoline-3-carbonitrile;

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-propyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile;

4-[(2,4-dichlorophenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3-quinolinecarbonitrile;

6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-4-[(3,4,5-trimethoxyphenyl)amino]quinoline-3-carbonitrile;

4-[(2-chloro-5-methoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

6-methoxy-4-[(5-methoxy-2-methylphenyl)amino]-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

4-[(2,4-dimethylphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

6-methoxy-4-[(5-methoxy-2,4-dimethylphenyl)amino]-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

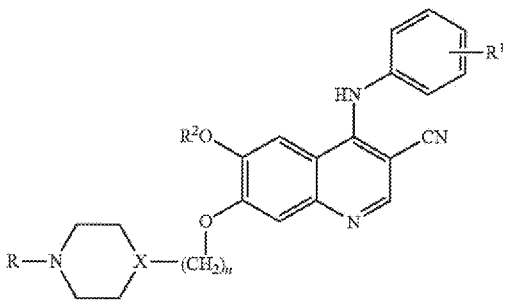
4-[(2,4-dichloro-5-ethoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile;

and pharmaceutically acceptable salts thereof.

The following experimental details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims that follow thereafter.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention are provided compounds of the structural formula I:



I

wherein:

n is an integer from 1-3;

X is N, CH, provided that when X is N, n is 2 or 3;

R is alkyl of 1 to 3 carbon atoms;

R^1 is 2,4-diCl, 5-OMe; 2,4-diCl; 3,4,5-tri-OMe; 2-Cl, 5-OMe; 2-Me, 5-OMe; 2,4-di-Me; 2,4-diMe-5-OMe, 2,4-diCl, 5-OEt;

R^2 is alkyl of 1 to 2 carbon atoms, and pharmaceutically acceptable salts thereof.

The compounds of this invention may be used for treating, preventing, or inhibiting CML. In a preferred embodiment the compounds are used as part of a pharmaceutical composition.

Pharmaceutically acceptable salts are those derived from such organic and inorganic acids as: acetic, lactic, carboxylic, citric, cinnamic, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, oxalic, propionic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, glycolic, pyruvic, methanesulfonic, ethanesulfonic, toluenesulfonic, salicylic, benzoic, and similarly known acceptable acids.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In a preferred embodiment, a straight chain or branched chain alkyl has 3 or fewer carbon atoms in its backbone.

The compounds may be provided orally, by intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, anal, vaginal, sublingual, uretheral, transdermal, intrathecal, ocular or otic delivery. In order to obtain consistency in providing the compound of this invention it is preferred that a compound of the invention is in the form of a unit dose. Suitable unit dose forms include tablets, capsules and powders in sachets or vials. Such unit dose forms may contain from 0.1 to 300 mg of a compound of the invention and preferably from 2 to 100 mg. In another embodiment the unit dosage forms contain 50 to 150 mg of a compound of the present invention. The compounds of the present invention can be administered orally. Such compounds may be administered from 1 to 6 times a day, more usually from 1 to 4 times a day. The effective amount will be known to one of skill in the art; it will

also be dependent upon the form of the compound. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity of the compound in bioassays and thus determine what dosage to administer.

The compounds of the invention may be formulated with conventional excipients, such as a filler, a disintegrating agent, a binder, a lubricant, a flavoring agent, a color additive, or a carrier. The carrier may be for example a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier. The carrier may be a polymer or a toothpaste. A carrier in this invention encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, acetate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

When provided orally or topically, such compounds would be provided to a subject by delivery in different carriers. Typically, such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, or glycols. The specific carrier would need to be selected based upon the desired method of delivery, for example, phosphate buffered saline (PBS) could be used for intravenous or systemic delivery and vegetable fats, creams, salves, ointments or gels may be used for topical delivery.

The compounds of the present invention may be delivered together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in treatment or prevention of neoplasm. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (for example, Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumins or gelatin to prevent absorption to surfaces, detergents (for example, TWEEN 20, TWEEN 80, PLURONIC F68, bile acid salts), solubilizing agents (for example, glycerol, polyethylene glycerol), anti-oxidants (for example ascorbic acid, sodium metabisulfate), preservatives (for example, thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (for example, lactose, mannitol), covalent attachment of polymers such as polyethylene glycol, complexation with metal ions, or incorporation of the compound into or onto particulate preparations of hydrogels or liposomes, micro-emulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of the compound or composition. The choice of compositions will depend on the physical and chemical properties of the compound capable of treating or preventing a neoplasm.

The compound of the present invention may be delivered locally via a capsule that allows a sustained release of the compound over a period of time. Controlled or sustained release compositions include formulation in lipophilic depots (for example, fatty acids, waxes, oils).

The present invention further provides a compound of the invention for use as an active therapeutic substance for treating, preventing, or inhibiting CML.

The present invention further provides a method of treating CML in humans, which comprises administering to the infected individual an effective amount of a compound or a pharmaceutical composition of the invention. The dose provided to a patient will vary depending upon what is being administered, the purpose of the administration, the manner of administration, and the like. A "therapeutically effective amount" is an amount sufficient to cure or ameliorate symptoms of CML.

The compounds of this may be delivered alone or in combination with other compounds used to treat CML. Such compounds include but are not limited to GLEEVEC, hydroxyurea, IFN-α, cytotoxic agents, 17-(Allylamino)-17-demethoxygeldanamycin or derivatives thereof, or wortmannin.

The compounds of this invention were prepared from: (a) commercially available starting materials (b) known starting materials which can be prepared as described in literature procedures or (c) new intermediates described in the schemes and experimental procedures herein. Compounds included in this invention can be prepared according to the synthesis routes disclosed in U.S. Pat. Nos. 6,002,008, and 6,780,996, such procedures are hereby incorporated by reference.

Reactions are performed in a solvent appropriate to the reagents and materials employed and suitable for the transformation being effected. It is understood by those skilled in the art of organic synthesis that the various functionalities present on the molecule must be consistent with the chemical transformations proposed. When not specified, order of synthetic steps, choice of protecting groups and deprotection conditions will be readily apparent to those skilled in the art. In addition, in some instances, substituents on the starting materials may be incompatible with certain reaction conditions. Restrictions pertinent to given substituents will be apparent to one skilled in the art. Reactions were run under inert atmospheres where appropriate.

The preparation of compounds of Formula I have been reported in the literature, [Boschelli, D. H., et. al., J. Med. Chem., 44, 3965 (2001)], Boschelli, D. H., et al., J. Med. Chem., 44, 822 (2001), Boschelli, D. H., et al., Bioorg. Med. Chem. Lett., 13, 3797 (2003), Boschelli, D. H., et. al., J. Med. Chem., 47, 1599 (2004), and Ye, F. et. al., 221*th National Meeting of the American Chemical Society*, San Diego, Calif. (April, 2001)].

This invention will be more fully described in conjunction with the following specific examples which are not to be construed as limiting the scope of this invention.

Materials and Methods:

Src kinase assay, homogeneous solution-based assay (Lance format)

Kinase Buffer:
50 mM Hepes pH 7.5
10 mM MgCl2
20 ug/ml BSA
0.001% Brij-35
(Prepare 2× kinase buffer for convenience:
100 mM Hepes, 20 mM MgCl2, add fresh 40 ug/ml BSA and 0.002% Brij)

Quench Buffer (to be added straight, 1:1, to reaction mix)
50 mM Hepes pH 7.5
60 mM EDTA
20 ug/ml BSA

Lance Detection Buffer and Plate Blocker:
50 mM Hepes pH 7.5
20 ug/ml BSA

Add EU-antibody PT66 (Perkin-Elmer) (1 nM) and APC-streptavidin (Perkin-Elmer) (4 ug/ml) for 100 ul/well just prior to using (add 100 ul to 50 ul rxn/50 ul quench for 200 ul final). 5×ATP=500 uM in water.

1. Rinse 96 well plate with 200 ul PBS. Preincubate 96 well black plate with 200 ul of 50 mM Hepes pH 7.5 with 20 ug/ml BSA for 10 minutes (lance detection buffer).
2. Kinase reaction takes place in a total volume of 50 ul kinase buffer in the 96 well plate. Use biotinylated substrate peptide at a final concentration of 2 uM, and src from Panvera at 5 ng per 50 ul reaction. The reaction is initiated by addition of 10 ul 5×ATP (final concentration 1×=100 uM) and carried out for 50 min @ 37° C. (per rxn: 25 ul 2× kinase buffer, 10 ul water, 5 ul diluted compound-10% DMSO/10 mM Hepes).
3. To stop kinase reaction add 50 ul of Quench buffer and shake for 30 s.
4. Add 100 ul of Lance detection buffer containing EU antibody and APC-strep. Add EU-antibody PT66 (1 nM) and APC-streptavadin (4 ug/ml) for 100 ul/well just prior to using (add 100 ul to 50 ul rxn/50 ul quench for 200 ul final). Incubate for 1 h @ room temp in the dark. Read Plate using the standard Lance protocol on the Wallac Victor.

Src Kinase Assay

Inhibitors of Src (partially purified enzyme preparation purchased from Upstate Biotechnologies, Lake Placid, N.Y.) tyrosine kinase activity are analyzed in an ELISA format. The Boehringer Mannheim Tyrosine Kinase Assay Kit (Roche Diagnostics, Basel, Switzerland) with a cdc2 substrate peptide containing Tyr15 is used for the assay. Horseradish Peroxidase (HRP)-conjugated anti-phosphotyrosine is used to detect phosphorylated peptide via a color reaction.

Reaction conditions: Five microliter aliquots of each compound prepared fresh at the time of the assay are added as a solution in 10 mM HEPES pH 7.5, 10% DMSO to the reaction well. Thirty-five microliters of reaction mix containing Src, buffer and peptide/bovine serum albumin mix are added to the compound wells and incubated at 30° C. for 10 minutes (reaction buffer: 50 mM TrisHCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM Na_3VO_4). The reaction is started by addition of 10 microliters of ATP (500 μM), incubated at 30° C. for 1 hour, and stopped by addition of 20 microliters of 0.5M EDTA. The reaction mixture with the phosphorylated peptide is then transferred to a streptavidin-coated microtiter plate and allowed to bind for 20 minutes. Unbound peptide and reaction mixture is decanted and the plate is washed with PBS six times. HRP-conjugated phosphotyrosine antibody supplied in the kit is incubated with the plate for one hour, then decanted. The plate is again washed with PBS six times. Substrate is added and absorbance at 405 nm is measured.

Alternatively, the assay performed essentially as described except a Delfia format (Perkin-Elmer) is used and Europium-conjugated phosphotyrosine antibody was used instead of HRP-conjugated phosphotyrosine antibody, Pierce Superblock was used in place of bovine serum albumin and 6 washes were employed after the kinase reaction and antibody binding. Europium fluorescence was used to monitor the extent of reaction.

Activity is determined as % inhibition as calculated by the formula: (1−Abs/Abs(max))×100=% inhibition. Where multiple concentrations of the test agent are used, an IC_{50} (concentration which gives 50% inhibition) can be determined. As shown in Table 2, compounds of the invention inhibit src kinase in vitro.

Homogeneous solution-based Abl kinase assay: Abl kinase activity was measured in a homogeneous assay format (Lance) where luminescence of a donor-acceptor complex bound to peptide phosphorylated by the kinase is measured in solution.

Biotinylated substrate peptide: Biotin-NH-KEEE-AIYAAPFAKKK-COOH (Synpep)

Kinase Buffer: 50 mM Hepes pH 7.5; 10 mM $MgCl_2$; 20 ug/ml BSA; 0.001% Brij-35; prepared as a 2× concentrate for convenience: 100 mM Hepes, 20 mM $MgCl_2$, add fresh 40 ug/ml BSA and 0.002% Brij-35

Quench Buffer to be added in equal proportions to the reaction mix: 50 mM Hepes pH 7.5; 60 mM EDTA; 20 μg/ml BSA

Lance Detection Buffer and plate blocker: 50 mM Hepes pH 7.5; 20 μg/ml BSA

Detection Mix: Antibody-APC reagent in Lance buffer to be added in equal proportions to the rxn mix/quench mix. Add 100 μL/well Lance detection buffer containing Eu-antibody PT66 (Perkin Elmer, AD0068; 1 nM final concentration in Lance detection buffer) and Streptavidin Surelight-APC (Perkin Elmer, CR130-100; 4 μg/mL final concentration in Lance detection buffer).

5×ATP=500 μM in water

Method:

1. Rinse 96 well plate with 200 μl PBS. Incubate 96 well black plate (Thermo LabSystems MicroFluor 2 black U-bottom microtiter plate; # 7205) with 200 μL of Lance detection buffer for 10 minutes.
2. Kinase reaction consists of a total volume of 50 μL kinase buffer/reaction in each well of a 96 well plate. Substrate peptide is present at a final concentration of 2 μM, and c-Abl from Panvera (c-Abl P3049) is included at 2.5 ng per 50 μL reaction. (per rxn: 25 μL 2× kinase buffer, 10 μL water, 5 μL diluted compound-10% DMSO/10 mM Hepes, pH 7.5). The reaction is initiated by addition of 10 μL 5×ATP (final concentration 1×=100 μM) and continued for 30 min @ 27° C.
3. Add 50 μL of Quench buffer to stop the kinase reaction.
4. Add 100 μL of Detection Mix.
5. Incubate for 30 min @ room temp in the dark. Measure luminescence at 665 nm on the Wallac Victor.

ANALYSIS OF RESULTS: % Inhibition=(*Cpm* (sample)−*Bkg*)/(*Cpm*(control)−*Bkg*))×100

The LSW data analysis plug-in for Excel (Model 63) is used to calculate IC50 values (y=Bmax/(1+(x/IC50)) Hyperbolic inhibition curve, Bmax to 0 (IC50).

These transformed Rat2 fibroblasts are used for the measurement of src dependent suspension growth.

Ultra-low cluster plates (Corning Costar, Acton, Mass.) are seeded with 10,000 cells per well on day 1. Alternatively, Ultra-low cluster plates (Costar 3474) treated with Sigmacote (Sigma, St. Louis, Mo.), rinsed with 70% ethanol, after drying in the hood, are seeded with 5000 cells. Compound is added in serial two-fold dilutions from 10 micromolar to 0.009 micromolar on day 2 and MTS reagent (Promega, Madison, Wis.) is added on day 5 (100 microliters of MTS/medium mix+100 microliters of medium already on the cells and the absorbance is measured at 490 nm. The results are analyzed as follows to yield an IC_{50} for proliferation (micromolar units) as follows: % inhibition=(Abs 490 nm sample−blank)/(Abs 490 nm no cmpd control−blank)×100%.

Alternatively relative cell numbers were determined by the CellTiter-Glo™ (Promega) method. All procedures were identical except that cell number was reduced to 1000 cells/well and CellTiter-Glo reagent was added instead of MTS reagent, with luminescence as the readout.

Anchorage Independent Src-Transformed Fibroblast Proliferation Assay

Rat2 fibroblasts stably transformed with a plasmid containing a CMV promoter controlled v-Src/HU c-Src fusion gene in which the catalytic domain of human c-Src gene as follows:

Cloning and plasmid constructions: the Prague C v-Src gene from pSrcHis (Wendler and Boschelli, Oncogene 4: 231-236; 1989) was excised with NcoI and BamHI, treated with T4 DNA polymerase, and cloned into the RI site of pTRE (Clontech) that had been rendered flush by treatment with T4 DNA polymerase. The PRC v-Src::hu c-Src fusion was created by replacing the Bgl2-XbaI fragment encoding the carboxyl terminal ~250 amino acids of v-Src with the Bgl2-XbaI fragment containing the v-Src::huc-Src fusion fragment (below). A partial clone of human c-Src was amplified from a breast cDNA library (InVitrogen) using the oligonucleotide pair 5'-CGCCTGGCCAACGTCTGCCCCACGTCCAAGCCGCAGACTCAGGGCCTG-3' (SEQ. ID NO: 1) and 5'-CCAACACACAAGCAGGGAGCAGCTGGGCCTGCAGGTACTCGAAGGTGGGC-3' (SEQ. ID NO: 2) and cloned into pCRScript (Stratagene). The catalytic domain of human c-Src in this clone was amplified with these oligonucleotides (fuses v-src nucleotide 734 to human c-Src nucleotide 742 and human c-Src nucleotide 1551 to v-src nucleotide 1543 in the v-Src and human c-Src ORFs). Two v-Src sequences were amplified by PCR (198 base pair v-src 5' fragment: 5'-GTGCCTATTGCCTCTCCGTTTCTGAC-3' (SEQ. ID NO: 3)(primer 1) to 5'-ACGTGGGGCAGACGTTGGCCAGGCG-3') (SEQ. ID NO: 4)(252 base pair 3' v-src fragment, 5'-CAGCTGCTCCCTGCTTGTGTGTTGG-3' (SEQ. ID NO: 5) (residues 1543-1567 in v-src ORF) to 5'-ATGAATTCTCTAGAGGAAGACGCCATCATATTCCAAGCAG-3' (SEQ. ID NO: 6) (residues 1769-1794 from v-src ATG with XbaI and EcoRI restriction sites added (primer 4)). Primers 1 and 4 were used to generate a three-fragment PCR amplification and fusion of the v-Src: human c-Src fusion fragment and the 5' and 3' fragments amplified from the Prague C v-Src gene and 3' untranslated region from Rous sarcoma virus. This reaction creates an in-frame v-Src::human c-Src gene fusion (amino acid residue V244 of v-Src to C248 of human c-Src on the amino terminal side and A517 of human c-Src to Q515 of v-Src). This gene fusion fragment encodes the carboxyl terminal one-third of the v-Src SH_2 domain and SH_2-catalytic domain linker fused to the human c-Src catalytic domain flanked by the v-Src carboxyl-terminal tail. A naturally occurring Bgl2 site near the 5' end of the fusion fragment and the engineered XbaI site at the 3' end of the fragment were used to excise fragment for creation of the full-length v-Src::human c-Src fusion gene as described above. The integrity of the constructs was confirmed by DNA sequencing. Similar methods were used to clone this gene into other expression plasmids such as pIRES (Clontech) for use in these studies.

Abl Kinase Assay.

Bacterially expressed Abl kinase was obtained from New England Biolabs. Kinase assays were performed in a DELFIA solid phase europium-based detection assay format (Perkin-Elmer). The peptide was as described in Dorsey et al. (46). Biotinylated peptide (2 μM) was bound to streptavidin coated microtitration plates (Perkin Elmer CC11-205) for 1.5 hour in 1 micrograms/ml ovalbumin in Phospate Buffered Saline (PBS). The plates were washed for 1 hour with PBS/0.1% Tween 80, followed by a PBS wash. The kinase reaction was incubated for 1 hour at 30° C. Abl kinase (10 units, NEB P6050L) was mixed with 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 80 μM EGTA, 100 μM ATP, 0.5 mM Na_3VO_4, 1% DMSO, 1 mM HEPES (pH 7) and 200 μg/ml ovalbumin. The reaction was stopped with EDTA at a final concentration of 50

mM. The DELFIA wash protocol suggested by the manufacturer (Perkin Elmer) was modified by extending wash times to reduce background. The reaction was monitored with Eu-labeled phosphotyrosine antibody (Perkin Elmer AD0040) and DELFIA Enhancement solution (Perkin Elmer 1244-105) according to manufacturer specifications.

Determination of Anti-Proliferative Activity of Compounds of Abl-Dependent Cells

A. Inhibition of v-Abl-dependent proliferation. Rat 2 cells infected with Abl-murine leukemia virus were grown and treated as described for the Src cell assay. All measurements were identical except for the cell type that Cell-Titer Glo (Promega) was used to monitor relative cell number. In this case, the reagent was used as recommended by the manufacturer and luminescence was measured on a Wallac Victor plate reader.

B. Inhibition of CML cell proliferation. KU812 and K562 cells were grown in RPMI1640 medium supplemented with 10% fetal calf serum and glutamine with 50 μg/ml gentamicin. Cells were plated at 1000-2000 cells per well on Day 0. On Day 1, compound was added such that the final DMSO concentration was no greater than 0.1%. On Day 4, Cell-Titer Glo was added according to manufacturer specifications and luminescence was determined on a Wallac Victor plate reader.

Results of these experiments are presented in Tables 1, 2 and 3 below.

Example 1

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile

mp 116-120° C.; MS (ES) m/z 530.2, 532.2 (M+1);

Example 2

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[3-(4-ethyl-1-piperazinyl)propoxy]-6-methoxy-3-quinolinecarbonitrile

mp 102-104° C.; MS (ES) m/z 544.3, 546.4 (M+1);

Example 3

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]-3-quinolinecarbonitrile

mp 165-167° C.; MS (ES) m/z 516.0, 518.2 (M+1);

Example 4

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[2-(4-ethyl-1-piperazinyl)ethoxy]-6-methoxy-3-quinolinecarbonitrile

mp 101-105° C.; MS (ES) m/z 530.4, 532.4 (M+1);

Example 5

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3-quinolinecarbonitrile

mp 200-202° C., MS 501.3 $(M+H)^+$, Analysis for $C_{25}H_{26}Cl_2N_4O_3$-0.8H_2O, Calcd: C, 58.21; H, 5.39; N, 10.86, Found: C, 58.19; H, 5.23; N, 10.67;

Example 6

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[2-(1-methylpiperidin-4-yl)ethoxy]-3-quinolinecarbonitrile

mp 190-191° C., MS 515.19 $(M+H)^+$, Analysis for $C_{26}H_{28}Cl_2N_4O_3$-1.0H_2O, Calcd: C, 58.53; H, 5.67; N, 10.50, Found: C, 58.65; H, 5.57; N, 10.34

Example 7

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(1-methylpiperidin-4-yl)propoxy]quinoline-3-carbonitrile

MP 144-145° C.; Mass spec. 529.2 (ES+);

Example 8

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[(1-ethylpiperidin-4-yl)methoxy]-6-methoxyquinoline-3-carbonitrile

MP 192-195° C.; Mass spec. 515.2 (ES+);

Example 9

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile

mp 137-138° C., MS 542.0 $(M-H)^-$, Analysis for $C_{27}H_{31}Cl_2N_5O_3$-0.6H_2O, Calcd: C, 58.40; H, 5.84; N, 12.61, Found: C, 58.31; H, 5.71; N, 12.43;

Example 10

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

mp 182-186° C., MS 513.0 $(M-H)^-$, Analysis for $C_{26}H_{28}Cl_2N_4O_3$-1.4H_2OCalcd: C, 57.76; H, 5.74; N, 10.36, Found: C, 57.65; H, 5.43; N, 10.15;

Example 11

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(4-ethylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile

mp 127-130° C., MS 558.3 $(M+H)^+$, Analysis for $C_{28}H_{33}Cl_2N_5O_3$-1.5H_2O, Calcd: C, 57.44; H, 6.20; N, 11.96, Found: C, 57.44; H, 6.24; N, 11.79;

Example 12

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[3-(1-methylpiperidin-4-yl)propoxy]quinoline-3-carbonitrile mp 148-151° C.

MS 543.2 $(M+H)^+$, Analysis for $C_{28}H_{32}Cl_2N_4O_3 \cdot 1.8H_2O$, Calcd: C, 58.39; H, 6.23; N, 9.73, Found: C, 58.40; H, 6.16; N, 9.64;

Example 13

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]quinoline-3-carbonitrile

mp 141-143° C., MS 530.2 (M+H)+, Analysis for $C_{26}H_{29}Cl_2N_5O_3$, Calcd: C, 58.87; H, 5.51; N, 13.20, Found: C, 58.48; H, 5.45; N, 12.95;

Example 14

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-ethoxy-7-[2-(1-methylpiperidin-4-yl)ethoxy]quinoline-3-carbonitrile

mp 174-176° C., MS 529.1 $(M+H)^+$, Analysis for $C_{27}H_{30}Cl_2N_4O_3$, Calcd: C, 61.25; H, 5.71; N, 10.58, Found: C, 61.40; H, 5.84; N, 10.35;

Example 15

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-propyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile

mp 97-101° C.; MS (ES) m/z 558.2, 560.2 (M+1);

Example 16

4-[(2,4-dichlorophenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3-quinolinecarbonitrile

mp 224-225° C., MS 469.0 (ES−);

Example 17

6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-4-[(3,4,5-trimethoxyphenyl)amino]quinoline-3-carbonitrile

mp>245° C.; HRMS (M+H)+ calculated 493.24455, found 493.24311;

Example 18

4-[(2-chloro-5-methoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

mp 106-108° C., MS 467.2 (ES+);

Example 19

6-methoxy-4-[(5-methoxy-2-methylphenyl)amino]-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

mp>250° C., MS 445.2 (ES−);

Example 20

4-[(2,4-dimethylphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

mp 190-191° C., MS 429.2 (ES−);

Example 21

6-methoxy-4-[(5-methoxy-2,4-dimethylphenyl)amino]-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

mp 160-162° C., MS 461.3 (ES+);

Example 22

4-[(2,4-dichloro-5-ethoxyphenyl)amino]-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinoline-3-carbonitrile

TABLE 1

ex	c-Abl Enzyme [a] IC_{50} nM	v-Abl cells IC_{50} nM	K562 IC_{50} nM	KU812 IC_{50} nM
1	1.1 (n = 2)	76 (n = 6)	20 (n = 19)	5.0 (n = 12)
3	not tested	440	48 (n = 2)	not tested
5	2.9 (n = 2)	617	39 (n = 3)	13.4 (n = 4)
6	2.9 (n = 2)	458	41	14.0
7	0.8 (n = 2)	185	18 (n = 4)	5.8 (n = 2)
16	16.0			
17	12.0			
18	3.5			
19	8.3			
20	38.0			
21	8.3			

TABLE 2

Tested in the Src enzyme assay, Examples 1-15
ELISA format, Examples 20-25 LANCE format

EXAMPLE	Src enzyme IC_{50} nM	Src cells IC_{50} nM
1	1.2	100
2	0.77	130
3	4.0	380
4	3.6	600
5	2.0	320
6	1.9	210
7	1.4	100
8	2.1	170
9	1.2	86
10	2.1	176
11	0.85	160
12	1.4	96
13	1.5	146
14	1.9	267
15	1.1	160
16	6.6	1400

TABLE 2-continued

Tested in the Src enzyme assay, Examples 1-15
ELISA format, Examples 20-25 LANCE format

EXAMPLE	Src enzyme IC_{50} nM	Src cells IC_{50} nM
17	8.3	1600
18	12	230
19	24	390
20	63	25000
21	13	510
22	230	

Compounds of formula I ("the compounds"), originally identified as a Src inhibitor, are shown here to be a potent antiproliferative and proapoptotic agent against CML cells in culture. The apoptotic activity of the compounds against CML cells in culture is mirrored by its activity in vivo against CML xenografts. K562 tumors regress in nude mice when the compounds are administered p.o. once a day. The Abl-inhibitory activity of the compounds is likely a major contributor to the antiproliferative activity of the compounds against CML cells. Tyrosine phosphorylation of Bcr-Abl is eliminated at concentrations of the compounds greater than 100 nm, which alone should be sufficient to inhibit the proliferation and survival of Bcr-Abl-dependent myeloid cells.

Nude mice with K562 xenografts were examined on days 11, 22, 36, and 43. Data is presented as a ratio of animals lacking detectable tumors relative to the number of animals per group. K562 tumors imbedded in Matrigel were staged in nude mice until tumors reached 200-300 mm^3. The compound of example 1 was administered p.o. in 0.4% methocel/0.5% Tween at 75 mg/kg once a day for 5 days (8 mice/group).

TABLE 3

Tumor-free survival of mice with K562 xenografts receiving various oral doses of example 1 for 5 days

	Day			
Dose	11	22	36	43
Vehicle	0/6			
150 mg/kg	8/8	8/8	8/8	8/8
100 mg/kg	8/8	7/8	7/8	7/8
75 mg/kg	7/8	6/8	6/8	6/8
50 mg/kg	6/8	5/8	4/8	4/8

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<223> OTHER INFORMATION: primer for human c-Src

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What is claimed is:

1. A pharmaceutical composition comprising a CML inhibiting amount of the compound 4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile.

* * * * *

EXHIBIT B

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

WYETH LLC, WYETH PHARMACEUTICALS LLC, PF PRISM C.V., PFIZER PHARMACEUTICALS LLC, and PFIZER PFE IRELAND PHARMACEUTICALS HOLDING 1 B.V. Plaintiffs, v. ALEMBIC PHARMACEUTICALS, LTD., ALEMBIC PHARMACEUTICALS, INC. , SUN PHARMACEUTICAL INDUSTRIES LIMITED and SUN PHARMACEUTICAL INDUSTRIES, INC., Defendants.	)	C.A. No. 16-1305 (RGA) CONSOLIDATED

REPLY EXPERT REPORT OF CRAIG W. LINDSLEY, PH.D.

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TABLE OF ABBREVIATIONS AND DEFINITIONS

Asserted Patents Discussed In This Report

'148 patent	U.S. Patent No. 7,417,148
'625 patent	U.S. Patent No. 7,919,625
Asserted Claims	Claim 7 of the '148 patent Claim 1 of the '625 patent

Table of References

Abbreviation	Citation	Exhibit Number
Boschelli 2001	Boschelli et al., Optimization of 4-Phenylamino-3-quinolinecarbonitriles as Potent Inhibitors of Src Kinase Activity, J. Med. Chem. 44:3965 (2001)	B
Boschelli 2002	Boschelli, et al., Dual Src/Abl Kinase Inhibitor Causes Regression of CML Xenografts in Nude Mice, Blood 100:786a (2002)	C
Donato 2003	Donato, et al., Use of c-Src Inhibitors Alone or in Combination with STI571 for the Treatment of Leukemia, WO 03/013540	D
Dorsey 2000	Dorsey et al., The Pyrido[2,3-d]pyrimidine Derivative PD180970 Inhibits p210 Bcr-Abl Tyrosine Kinase and Induces Apoptosis of K562 Leukemic Cells, Cancer Research, 60, 2127-3131 (2000)	E
Golas 2003	Golas et al., SKI-606, a 4-Anilino-3-quinolinecarbonitrile Dual Inhibitor of Src and Abl Kinases, Is a Potent Antiproliferative Agent Against Chronic Myelogenous Leukemia Cells in Culture and Causes Regression of K562 Xenografts in Nude Mice, Cancer Res. 63:375 (2003)	F
Kantarjian 2006	Kantarjian et al., Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL, New Engl J Med 354(24):2542-51 (2006)	G
Lionberger 2000	Lionberger et al., Transformation of Myeloid Leukemia Cells to Cytokine Independence by Bcr-Abl Is Suppressed by Kinase-defective Hck, J. Biol. Chem. 275:18581-18585 (2000)	H

Nimmanapalli 2002	Nimmanapalli et al., Molecular Characterization and Sensitivity of STI-571 (Imatinib Mesylate, Gleevec)-resistant, Bcr-Abl-Positive, Human Acute Leukemia Cells to SRC Kinase Inhibitor PD180970 and 17-Allylamino-17-Demethoxygeldanamycin, Cancer Res. 62:5761-5769 (2002)	I
Norman 2016	Norman, Drugs, Devices, and the FDA: Part 1: An Overview of Approval Processes for Drugs, JACC : Basic To Translational Science Vol. 1, No. 3, 170-179 (2016)	J
SPRYCEL Prescribing Information	SPRYCEL Prescribing Information, available at http://packageinserts.bms.com/pi/pi_sprycel.pdf	K
Talpaz 2006	Talpaz et al., Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias, New Engl J. Med. 354(24):2531-41 (2006)	L
TASIGNA Prescribing Information	TASIGNA Prescribing Information, available at https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/tasigna.pdf	M
Tatton 2003	Tatton et al., The Src-selective Kinase Inhibitor PP1 Also Inhibits Kit and Bcr-Abl Tyrosine Kinases, J. Biol. Chem. 278(7):4847-4853	N
Warmuth 1997	Warmuth et al., The Src Family Kinase Hck Interacts with Bcr-Abl by a Kinase-Independent Mechanism and Phosphorylates the Grb2-Binding Site of Bcr, J. Biol. Chem. 272(52):33260-33270 (1997)	O
Warmuth 2003	Warmuth et al., Dual-Specific Src and Abl Kinase Inhibitors, PP1and CGP76030, Inhibit Growth and Survival of Cells Expressing Imatinib Mesylate-Resistant Bcr-Abl Kinases, Blood 101:664-672 (2003)	P
Williams 1998	Williams, et al., Insights into Src kinase functions: structural comparisons, Trends in Biochemical Sciences, 23(5):179-184 (1998)	Q
Wisniewski 2002	Wisniewski, et al., Characterization of Potent Inhibitors of the Bcr-Abl and the c-Kit Receptor Tyrosine Kinases, Cancer Res. 62:4244 (2002)	R

Wolff 2003	Wolff et al., Office of Laboratory Animal Welfare Frequently Asked Questions About the Public Health Service Policy on Humane Care and Use of Laboratory Animals, 32 Lab Animal 33 (2003)	S
Zimmerman 1997	Zimmerman et al., Potent and Selective Inhibitors of the Abl-Kinase: Phenylaminopyrimidine (PAP) Derivatives, 7 Bioorganic & Med. Chemistry Letters 187 (1997)	T

I. INTRODUCTION

1. I, Craig W. Lindsley, Ph.D., submit this reply expert report on behalf of Defendants Alembic Pharmaceuticals, Ltd., Alembic Pharmaceuticals, Inc., Sun Pharmaceuticals Industries Limited and Sun Pharmaceutical Industries, Inc. (collectively, "Defendants") in the above-captioned litigation brought by Wyeth LLC, Wyeth Pharmaceuticals Inc., PF Prism C.V., Pfizer Pharmaceuticals LLC, and Pfizer PFE Ireland Pharmaceuticals Holding 1 B.V. ("Plaintiffs"). I am the same Craig W. Lindsley who submitted the Opening Report of Craig W. Lindsley, Ph.D., on April 24, 2019 ("Lindsley Opening Report") and the Responsive Report of Craig W. Lindsley, Ph.D., on May 31, 2019 ("Lindsley Rebuttal Report").

2. I have been asked by counsel for Defendants to review the Expert Report of Mark Levis, M.D., Ph.D. (the "Levis Report"), the Responsive Expert Report of Bernhardt L. Trout, Ph.D. (the "Trout Report"), the Responsive Expert Report of Mark A. Murcko, Ph.D. (the "Murcko Report"), and the Expert Report of Neil Shah, M.D., Ph.D. (the "Shah Report") (collectively, "Plaintiffs' Expert Reports"), all served on May 31, 2019, and references cited therein, and to respond to certain assertions therein.

3. In this reply report, I cite representative paragraphs from the above-mentioned Reports that address the issues discussed. My lack of response to any particular assertion does not mean that I necessarily agree with the assertion.

4. In addition to Plaintiffs' Expert Reports and the materials cited therein, as well as the materials cited in this report, I have set forth in Exhibit A to this report materials I have considered in forming the opinions expressed in this report. I have also relied upon my knowledge, education, and training as described in section II of my Opening Report, which I incorporate herein by reference, and the materials identified in Exhibit A to my Opening Report.

5. I have based my opinions and analysis on documents and information available to me at the time I signed this report. If and when any new evidence or arguments arise, I reserve the right to supplement or modify my opinions to reflect such evidence or arguments.

6. In the event that Plaintiffs submit any response to this expert report, I reserve the right to respond to any issues raised by such a response.

7. If called to testify, my testimony may include an explanation of the scientific principles that underlie the opinions expressed in this report.

8. I reserve the right to make and use demonstratives to help explain my opinions.

II. SUMMARY OF OPINIONS

9. In my Opening Report, I opined that claim 7 of U.S. Patent No. 7,417,148 ("the '148 patent") is invalid as not enabled, lacking sufficient written description, and obvious over the prior art, and claim 1 of U.S. Patent No. 7,919,625 ("the '625 patent") is invalid as not enabled, lacking sufficient written description, indefinite, and anticipated by and obvious over the prior art. Nothing in Plaintiffs' Expert Reports changes my opinions as set forth in my Opening Report. In particular:

10. I disagree with Dr. Levis that claim 7 of the '148 patent and claim 1 of the '625 patent satisfy the requirements of 35 U.S.C. § 112.

11. I disagree with Dr. Trout that claim 1 of the '625 patent is not anticipated by Boschelli 2001.

12. I disagree with Drs. Murcko and Shah that claim 7 of the '148 patent and claim 1 of the '625 patent are not rendered obvious by the prior art.

13. I disagree with Dr. Shah that any long-felt need or unexpected results demonstrate nonobviousness of claim 7 of the '148 patent or claim 1 of the '625 patent.

III. BACKGROUND AND THE ASSERTED PATENTS

14. I address below certain statements in Plaintiffs' Expert Reports regarding the technical background relevant to the Asserted Patents. It should be understood that the fact that I have not addressed a particular statement in Plaintiffs' Expert Reports does not necessarily mean I agree with such statement.

A. LEVIS REPORT

15. I disagree with Dr. Levis's statement that clinical trials of imatinib would provide guidance for a POSA in conducting studies of bosutinib, a structurally unrelated BCR-ABL inhibitor. Levis Report, ¶ 68. A POSA would have understood that the way in which a particular compound may be effectively administered (*i.e.*, dosage form, route of administration, amount, and frequency) is a function of a number of compound-specific variables, including its pharmacokinetic properties, solubility, toxicity, potency, etc. As of November 6, 2003, there was no art characterizing these properties for bosutinib; therefore, a POSA would not have had any information in order to compare bosutinib's properties with imatinib, and could not have a basis for determining whether the properties of these two compounds were comparable. A POSA also would not have expected these properties to be the same for the two compounds. Therefore, a POSA would not have been guided by imatinib clinical trials in determining how to effectively administer bosutinib.

16. For similar reasons, I disagree with Dr. Levis's statement that the imatinib clinical trials would have "provided guidance on dosing CML patients, showing that 400 mg of imatinib per day was the preferred dose to treat chronic phase CML patients but that the dose could be increased if the patients failed to achieve cytogenetic response." Levis Report, ¶ 86. The dose of imatinib would not have provided guidance on how another drug should be dosed to treat CML, regardless of whether both drugs were known to be BCR-ABL inhibitors. For example, despite

the experience with imatinib, a phase I dose escalation study performed for the BCR-ABL kinase inhibitor dasatinib examined dasatinib administration at a range 15 to 240 mg per day—a dose much lower than the 400 mg/day determined to be therapeutically effective for imatinib. *See* Talpaz *et al*., *Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias,* New Engl J. Med. 354(24):2531-41 (2006). Today, dasatinib (marketed as SPRYCEL®) is administered to chronic phase CML patients at a dose of 100 mg once daily, and for accelerated or blast phase CML patients at a dose of 140 mg once daily. *See* SPRYCEL Prescribing Information, *available at* http://packageinserts.bms.com/pi/pi_sprycel.pdf. Meanwhile, a phase I dose escalation study of the BCR-ABL kinase inhibitor nilotinib examined doses at 50 mg, 100 mg, 200 mg, 400 mg, 600 mg, 800 mg, and 1200 mg once daily and at 400 mg and 600 mg twice daily. *See* Kantarjian *et al.*, *Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL,* New Engl J Med 354(24):2542-51 (2006). Today, nilotinib (marketed as TASIGNA®) is administered to chronic phase CML patients at a dose of 300 mg *twice* daily, for a total of 600 mg per day, and is administered to imatinib-resistant CML patients at a dose of 400 mg twice daily, for a total of 800 mg per day. *See* TASIGNA Prescribing Information, *available at* https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/tasigna.pdf. The difference between the selected dosages above and imatinib's dosage indicate that a POSA would not have been guided by the dose for imatinib, even specifically a BCR-ABL kinase-inhibiting CML drug.

17. I disagree with Dr. Levis's statement that the Wyeth researchers who published the data in Golas 2003 did not show that bosutinib acted as anything other than a BCR-ABL inhibitor. Levis Report, ¶ 99. Because Src family kinases act downstream of BCR-ABL (*see, e.g.*, Lionberger 2000, Warmuth 1997), inhibition of BCR-ABL would necessarily inhibit

activation by BCR-ABL of Src family kinases. Therefore, the capability of bosutinib to inhibit Src family kinases would be masked by its ability to also inhibit BCR-ABL in these studies. A POSA would thus have expected consistency – not discordance, as alleged by Dr. Levis – among the CML cell line proliferation assays and the results in the phosphorylation assays for a dual Src/BCR-ABL inhibitor like bosutinib. However, if the BCR-ABL gene in a particular CML cell line were mutated in such a way that the BCR-ABL tyrosine kinase no longer responds to bosutinib, a POSA would also reasonably expect bosutinib to inhibit CML proliferation through its ability to inhibit Src family kinases.

18. I disagree with Dr. Levis's characterization of dose-finding studies as "routine." Levis Report, ¶ 101. Dr. Levis acknowledges that both *in vitro* and *in vivo* toxicity studies would have needed to be carried out before a drug could even be tested in a human. *Id.* He further acknowledges that there were many methods a POSA could have used to identify a first-in-human dose based on these studies. *See, e.g.*, Levis Report, ¶¶ 105-112. These methods involved estimates and were left to the discretion of the researcher, and would need to have been adjusted as more data were gathered. A POSA would also have needed to carry out dose escalation studies in humans to further evaluate its safety profile. Again, the specific doses used in these studies were similarly subject to the researcher's discretion. With the data collected from these extensive studies, a POSA would finally have been in a position to select potentially-effective dosing regimens to administer to patients in order to determine an appropriate therapeutic dose, with the selection of specific doses left to the discretion of the researcher. The

extensive experimentation involved in ascertaining a therapeutically effective dose is anything but "routine."[1]

19. I disagree with Dr. Levis's assertion that Wyeth's development of a therapeutically effective dosing regimen of bosutinib for human administration was "routine." *See* Levis Report at ¶¶ 114-41. To the contrary, the many years of experimentation that Wyeth needed to perform in order to determine a therapeutically effective dose of bosutinib for human administration—starting with the early animal toxicity studies in 2003 and continuing through the phase 1/2 study results reported in 2011—demonstrates that a POSA would have had to conduct undue experimentation in order to practice the asserted claims.

20. I disagree with Dr. Levis's opinion that "POSA reading the '148 patent would have known that physicians treating CML patients with BCR-ABL1 inhibitors as of November 2003 used standard criteria to measure the efficacy of the drug." Levis Report, ¶ 181. Instead, a POSA in November 2003 would have been aware of only one FDA-approved BCR-ABL inhibitor and would not have known what physicians did to measure the efficacy of other BCR-ABL inhibitors, because no other BCR-ABL inhibitors were being used in the clinic.

B. TROUT REPORT

21. To the extent Dr. Trout suggests that the purpose of preclinical development of a drug is to evaluate the drug for development of a pharmaceutical composition of that drug, I disagree with that statement. Trout Report, ¶ 48. Instead, the purpose of preclinical development of a drug is to develop a drug for therapeutic administration to a human being; pharmaceutical compositions of that drug can be administered to animals during preclinical

[1] As explained in my Opening Report, to the extent determining an appropriate dose of bosutinib would, in fact, have been "routine" based on the disclosure of the patents-in-suit, then such determination would likewise have been "routine" from the prior art.

development in pursuit of that goal. In fact, because preclinical development of a drug includes administration of that drug to animals such as rats and dogs, and because drug compounds need to be formulated with at least a carrier and/or some excipient for administration—they usually cannot be administered to a mammal in pure form—preclinical development of a drug necessarily requires administration of a pharmaceutical composition of that drug.

22. I disagree with Dr. Trout's statement that "[t]he lead discovery phase generally does not encompass making pharmaceutical compositions." Trout Report, ¶ 49. While the lead discovery phase of drug development does not generally encompass administering pharmaceutical compositions to human beings, it does involve initial dosing of animals with pharmaceutical compositions of drug candidates in order to determine whether those drug candidates should be further developed for human administration.

23. I disagree with Dr. Trout's statement that the work done during preclinical development "form[s] the basis for development of a pharmaceutical composition." Trout Report, ¶ 50. Instead, the work done during preclinical development serves to inform a POSA if, and how, a particular drug candidate can be safely and effectively administered to a human.

C. MURCKO REPORT

24. I disagree with Dr. Murcko's assertion that a POSA would not have been able to reasonably predict whether a known inhibitor of a Src tyrosine kinase would have inhibitory activity against BCR-ABL tyrosine kinase. *See* Murcko Report at Section VII(L). By 2003, a POSA would have known that the structure of Abl kinase was very similar to Src family kinases, not only in the primary sequence of the ATP-binding pocket of protein tyrosine kinases, but in the overall structure as well. *See, e.g.,* Williams 1998 at 184 ("The Abl, Btk and Csk kinase families are made up of SH3, SH2 and kinase domains that have the same topology as, and show high sequence similarity to, Src"). Thus, a compound with known inhibitory activity against a

Src family protein tyrosine kinase was very likely to also have inhibitory activity against the Abl kinase.

25. Dr. Murcko cites an article published in 1997 by Zimmerman et al. (Murcko Report, Ex. CC) purportedly illustrating that structurally related compounds "demonstrate[] widely varied and unpredictable inhibition activity against different tyrosine kinases, including v-Abl kinase and SRC." Murcko Report, ¶ 100. However, a POSA would have understood from Table 2 of the Zimmerman reference that a potent Src inhibitor would likely also be a potent Abl inhibitor. In fact, every compound in Table 2 that inhibited Src also inhibited Abl. Zimmerman at Table 2. Furthermore, every compound in Table 2 with an IC_{50} of less than 50 μM for Src kinase had an IC_{50} of less than 5 μM for Abl kinase, suggesting that more a more potent Src inhibitor would likely be a more potent Abl inhibitor. *Id*. Therefere, a POSA would have expected that bosutinib, a known potent Src inhibitor, would also very likely strongly inhibit BCR-ABL.

IV. THE ASSERTED CLAIMS ARE INVALID FOR FAILURE TO SATISFY THE REQUIREMENTS OF 35 U.S.C. § 112

A. Lack of Written Description

1. Claim 7 of the '148 Patent

26. I disagree with Dr. Levis's opinion that a POSA reading the disclosures of the asserted patents would find that these disclosures satisfy the written description requirement for claim 7 of the '148 patent. Claim 7 of the '148 patent requires administering a "therapeutically effective amount" of bosutinib. Accordingly, to be in possession of the subject matter of either claim, the named inventors must have known how much bosutinib to administer, in what form, by what route, and how often, in order to treat CML. However, Dr. Levis acknowledges that the asserted patents do not disclose a specific dosing regimen for use in humans. Levis Report, ¶

171. A POSA would have understood, as Dr. Levis acknowledges, that the named inventors "had not yet administered bosutinib to humans or determined a specific dosing regimen for use in humans." *Id*.

27. Dr. Levis further acknowledges that "appropriate human dosing can only be determined through human clinical trials," and the patents provide no indication that the named inventors had carried out such human clinical trials. Levis Report, ¶¶ 101, 171. Dr. Levis acknowledges that the patents instead expressly leave the task of determining a therapeutically effective amount to the POSA. Levis Report at ¶ 172 (quoting '148 patent at 3:66-4:4). Accordingly, a POSA would not have understood from reading the disclosures of the asserted patents that the named inventors were in possession of a method of providing a "therapeutically effective amount" of bosutinib.

28. I further disagree with Dr. Levis's assertion that the FDA-approved dose amounts of 400 mg and 500 mg bosutinib once daily are disclosed by the asserted patents. The specifications recite a maximum unit dosage of 300 mg bosutinib. '148 patent at 3:57-64; '625 patent at 3:60-66. While the specification indicates that this dose may be administered multiple times a day, 400 mg taken *once* daily is not the same as 100 mg taken four separate times throughout the day due to differences in drug absorption of the different administration schedule, even if both schedules result in taking 400 mg of bosutinib in a 24-hour time span. Indeed, these two different regimens may provide completely different efficacy and toxicity. Therefore, the asserted patents do not disclose 400 mg or 500 mg bosutinib administered once daily.

29. I further disagree with Dr. Levis's opinion that the asserted patents disclose a therapeutically effective dosage form or schedule of bosutinib. Levis Report, ¶ 177. As Dr. Levis acknowledges, the patent specifications disclose a broad range of possible dosage forms

and schedules for bosutinib. There is nothing in these disclosures that indicate that the named inventors knew bosutinib should be formulated into a solid dosage form for oral administration, or how much or how frequently bosutinib should be administered to be therapeutically effective. For example, the '148 patent specification provides an incredibly broad list of possible routes of administration, including "orally, by intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, anal, vaginal, sublingual, uretheral, transdermal, intrathecal, ocular or otic delivery" ('148 patent at 3:50-54) and then provides an even broader list of potential materials to develop a dosage form (*id*. at 4:3-53). The specification also states that "unit dose forms may contain from 0.1 to 300 mg of a compound of the invention and preferably from 2 to 100 mg. In another embodiment the unit dosage forms contain 50 to 150 mg of a compound of the present invention. The compounds of the present invention can be administered orally. Such compounds may be administered from 1 to 6 times a day, more usually from 1 to 4 times a day." *Id*. at 58-64.

30. This laundry list of routes of administration, dosage forms, and schedules is so broad that a POSA would not have understood that the named inventors were in possession of an actual method of administration that was, in fact, therapeutically effective. For example, even though "solid oral dosage forms are commonly used and are generally most convenient for patients" (Levis Report ¶ 177), some compounds (including standard chemotherapies such as cisplatin) are not administered orally and are best administered intravenously. A POSA would not have known whether this would also be true of bosutinib, much less whether the named inventors had determined whether this was true of bosutinib. Moreover, even if a method for effectively administering bosutinib would have been obvious ***to a POSA*** from the information provided in the patent, nothing in the patent suggests that ***the named inventors*** had, in fact,

discovered how to do so. Indeed, to the extent Dr. Levis is correct that a POSA would have understood that bosutinib should be administered orally, then the fact that the patent merely identifies oral administration as only one of many possible methods would have confirmed to a POSA that the named inventors were not, in fact, in possession of the claimed method.

31. I also disagree with Dr. Levis's opinion that "a POSA would know that BCR-ABL inhibitors can be administered to patients orally because Gleevec was marketed as a solid, oral dosage form." Levis Report, ¶ 177. As I explained above, a POSA would not have understood how to properly administer bosutinib based on prior experience with Gleevec, a structurally unrelated molecule, simply because both were known to be BCR-ABL inhibitors. It is the physiochemical and pharmacokinetic properties of a drug that determine the therapeutically effective dosage form, route of administration, and appropriate human dosing schedule, and the asserted patents do not disclose any data that would allow a POSA to make such determinations.

2. Claim 1 of the '625 Patent

32. I also disagree with Dr. Levis's opinion that a POSA reading the disclosures of the asserted patents would find that these disclosures satisfy the written description requirement for claim 1 of the '625 patent.

33. *First*, I disagree with Dr. Levis that the phrase "CML inhibiting amount" in claim 1 of the '625 patent refers to a "therapeutically effective amount." Levis Report, ¶ 189. As I explain further below in Section IV.C regarding indefiniteness of claim 1 of the '625 patent, a POSA would not have understood what it means to "inhibit" a disease such as CML, would have considered "CML inhibiting amount" to necessarily mean something different from "therapeutically effective amount" because those are distinct phrases, and a POSA would not

have understood it to be possible for a pharmaceutical composition to have a "CML inhibiting amount."

34. *Second*, even assuming that the phrases "therapeutically effective" in claim 7 of the '148 patent and "CML inhibiting" in claim 1 of the '625 patent mean the same thing, for the same reasons I disagree with Dr. Levis regarding the written description of claim 7 of the '148 patent, I disagree regarding his opinion regarding written description of claim 1 of the '625 patent.

35. *Third*, to the extent that Dr. Trout is correct (he is not) that a "pharmaceutical composition" or a "pharmaceutically acceptable composition" requires the use of active pharmaceutical ingredient at a certain level of purity, USP-NF grade excipients, and adherence to particular procedures to ensure the sterility, pH, form of dextrose, and toxicity of a particular formation, the '625 patent does not either specify such requirements or disclose their use in either the specification or the examples. Thus, to the extent that Boschelli 2001 does not anticipate claim 1 of the '625 patent, claim 1 of the '625 lacks sufficient written description support.

B. Lack of Enablement

1. Claim 7 of the '148 Patent

36. I disagree with Dr. Levis's opinion that a POSA reading the disclosures of the asserted patents would find that these disclosures satisfy the enablement requirement for claim 7 of the '148 patent.

37. In particular, I disagree that a POSA could have used the information provided in the '148 patent to administer a therapeutically effective amount of bosutinib to treat CML without undue experimentation. Levis Report, ¶¶ 179, 192. A POSA would have understood that a "therapeutically effective amount" of bosutinib, defined by the patent specification to

mean "an amount sufficient to cure or ameliorate symptoms of CML," requires selecting a dose of bosutinib that avoids a certain level of toxicity to the human patient while meeting a sufficient level of efficacy in treating CML in order to be considered *therapeutically* effective. That is, to take an extreme example, a POSA would have understood that an amount of bosutinib that cures CML by destroying all CML cells, but also kills the patient, would *not* be considered a "therapeutically effective amount" of bosutinib, even if all CML cells were technically eliminated. Therefore, a POSA would have understood that certain data regarding toxicity and efficacy were required in order to practice claim 7 of the '148 patent. Dr. Levis notably agrees that such data would have been required before bosutinib could be effectively administered to humans. Levis Report, ¶ 101.

38. Furthermore, I disagree with Dr. Levis's opinions that claim 7 of the '148 patent is enabled based on the availability of previously disclosed methods to determine a therapeutically effective dose. Levis Report, ¶¶ 181-186. Claim 7 of the '148 patent requires administering a "therapeutically effective amount" of bosutinib. Therefore, claim 7 is only enabled if a POSA could determine a "therapeutically effective amount" of bosutinib without undue experimentation. Whether a POSA would have been able to test whether a known dosing regimen was therapeutically effective (Levis Report, ¶ 181), or whether a POSA was aware of possible approaches by which one *might* discover an appropriate dose for treatment of CML in humans (Levis Report, ¶¶ 182-186), means only that a POSA may have been able to devise a research plan for identifying an appropriate dose, not that the experimentation needed to actually achieve that goal was not undue, or even that a POSA would have understood such a result was actually achievable.

39. In fact, as Dr. Levis points out, Wyeth conducted studies for years before determining an appropriate therapeutically effective dose of bosutinib to treat CML. *See generally*, Levis Report, Section VII.B. Wyeth started with animal toxicity and pharmacokinetic studies with bosutinib in 2003, which were not disclosed in either the '148 or '625 patents, and conducted single dose studies in mice and rats, followed by repeat-dose studies of orally administered bosutinib in a seven-day rat study, a 10-day dog study, and a four-week study in rats and dogs. *Id*. at ¶ 116. Notably, the starting clinical dose for human administration was not determined until after these studies were conducted. *Id*. at ¶ 117.

40. Wyeth then conducted a phase I study of bosutinib in patients with advanced malignant solid tumors, not CML specifically, and started with a dose of 50 mg/day. *Id*. at ¶ 118. This study was conducted in two parts: dose escalation, and dose expansion. *Id*. at ¶¶ 120-121. The dose escalation part of the study examined doses ranging from 50 mg/day to 1000 mg/day. *Id*. at ¶ 119. Pharmacokinetic analyses were also conducted on patients' blood samples on days 1 and 15 after continuous daily administration. *Id*. at ¶ 123.

41. After this study, Wyeth began yet another clinical trial in 2006[2], this time a phase 1/2 study that again began with a dose escalation study, examining doses ranging from 400 mg/day to 600 mg/day. *Id*. at 124-125; *see also* NCT00261846. For this first part of this study, Wyeth was still evaluating the safety of the given doses over the course of 28 days to establish the maximum tolerated dose. Levis Report, ¶ 125.

42. The second part of the study enrolled 271 patients in addition to the 18 patients enrolled in the first part of the study, and evaluated cytogenetic, molecular, and hematologic

[2] This study began on January 18, 2006 and had a primary completion date of September 25, 2009. *See* clinical trial NCT00261846, *available at* https://clinicaltrials.gov/ct2/show/NCT00261846?term=NCT00261846&rank=1.

responses for *two years*. *Id*. at ¶¶ 127-128. After two years, assessments were performed every six months. *Id*. at ¶ 128.

43. Wyeth, which was acquired by Pfizer in 2009, ultimately relied on the data obtained from this study (clinical trial NCT00261846) to support its application for FDA approval of bosutinib for the treatment of CML.

44. Given the quantity of experimentation that Wyeth had to conduct in order to determine a therapeutically effective dose of bosutinib, and the lack of data and direction provided by the specification of the ’148 patent (including the lack of any animal toxicity data), a POSA would have needed to conduct undue experimentation in order to practice claim 7 of the ’148 patent.[3]

2. Claim 1 of the ’625 Patent

45. Assuming that a “CML inhibiting amount” of bosutinib as claimed in claim 1 of the ’625 patent means a “therapeutically effective amount” of bosutinib as claimed in claim 7 of the ’148 patent, then for the same reasons I disagree with Dr. Levis regarding enablement of claim 7 of the ’148 patent, I disagree regarding his opinion regarding enablement of claim 1 of the ’625 patent.

C. Claim 1 of the ’625 Patent Is Indefinite

46. I disagree with Dr. Levis’s opinion that claim 1 of the ’625 patent informs a POSA with reasonable certainty about the scope of the claimed invention because, as I explained in my Opening Report (Lindsley Opening Report, Section VIII.B) and as explained below, a POSA would not have understood what a “CML inhibiting amount” means.

[3] To the extent that undue experimentation was not required in order to practice claim 7 of the ’148 patent or claim 1 of the ’625 patent, these claims are rendered obvious by the prior art.

47. *First*, a POSA would not have known what it means to "inhibit" CML. A POSA would have understood that a drug may inhibit the activity of a particular kinase or modulate a particular signal transduction pathway, or in the case of less targeted therapies, a drug may demonstrate a more wide-ranging effect, such as general cytotoxicity. However, no drug "inhibits" a particular disease. Instead, a given drug implements a particular effect that physicians take advantage of in order to treat a disease, but a POSA would not have understood what it means to "inhibit" the disease itself.

48. *Second*, I disagree with Dr. Levis's opinion that a POSA reading the '625 patent would have understood the claim term "CML inhibiting amount" as another way of saying "therapeutically effective amount." Levis Report, ¶ 195. Dr. Levis provides no support for his conclusory assertion, and contrary to his opinion, these are different phrases, and therefore necessarily mean different things. Because the named inventors used the phrase "therapeutically effective amount" in claim 7 of the '148 patent, they would have known to use the same phrase if that is what the named inventors meant for claim 1 of the '625 patent; but instead, the named inventors chose a different phrase. Thus, a POSA would have understood that the named inventors were referring to something else. Also, while the phrase "therapeutically effective" is defined within the patent specification, the phrase "CML inhibiting" is not. For reasons I explained above and in my Opening and Responsive Reports, because there is no plain and ordinary meaning for the phrase "CML inhibiting amount," a POSA would not have known what that phrase means, even if the POSA understood the phrase "therapeutically effective amount."

49. *Third*, and as I explained in my Opening Report, even assuming that a POSA understood "CML inhibiting amount" to mean a "therapeutically effective amount" of bosutinib,

a POSA would not have understood a pharmaceutical composition to be capable of comprising a "CML inhibiting amount" of bosutinib.

50. Dr. Levis inexplicably deviates from his own definition and asserts that "a pharmaceutical composition comprising a CML inhibiting amount" does not even require a "therapeutically effective amount" in the composition. Levis Report, ¶ 196. Instead, he alleges that a "CML inhibiting amount" is actually "a dose that would be therapeutically effective when administered on an ongoing basis to inhibit CML in a patient." *Id*. In addition to being inconsistent with the language of the claim itself, there are two fundamental problems with this interpretation.

51. First, claim 1 of the '625 patent does not specify over what period of time a composition must be effectively administered when determining whether it has a "CML inhibiting amount" of bosutinib. Thus, for example, a pharmaceutical composition comprising a 100 mg dose of bosutinib might not be therapeutically effective if administered once daily, even if administered over 24 weeks. However, the same pharmaceutical composition might be therapeutically effective if administered four at a time, once daily, so that a total dose of 400 mg of bosutinib were delivered as a daily dose. Because claim 1 of the '625 patent is not limited to any particular schedule or frequency of administration, whether or not a particular composition meets the "CML inhibiting amount" limitation would depend upon how infringement is measured (*i.e.*, the frequency with which the composition is administered to determine whether it effectively treats CML), and claim 1 of the '625 patent fails to inform the POSA with reasonable certainty about the scope of the claimed invention.

52. Second, to the extent Dr. Levis intends to suggest that a composition meets the "CML inhibiting amount" limitation so long as it can be administered in some way so as to treat

CML, such an interpretation effectively eliminates the limitation from the claim. That is because a composition containing ***any*** amount of bosutinib could effectively treat CML if administered on an appropriate schedule. For example, a composition containing 400 mg of bosutinib can effectively treat CML if administered once daily, and thus would have a "CML inhibiting amount" under this construction. But so too would a composition containing 40 mg of bosutinib, because ten such compositions could be administered at one time, once daily. Or a composition containing 4 mg of bosutinib, of which 100 could be administered once daily. This interpretation therefore renders the term "CML inhibiting amount" superfluous.

53. Accordingly, claim 1 of the '625 patent is indefinite even if interpreted to mean "therapeutically effective amount" as advocated by Dr. Levis.

V. CLAIM 1 OF THE '625 PATENT IS INVALID AS ANTICIPATED BY BOSCHELLI 2001

54. As I explained above, claim 1 of the '625 is indefinite and therefore cannot be construed. However, to the extent "CML inhibiting amount" can be construed as "therapeutically effective amount," and assuming a POSA would have understood the scope of claim 1 of the '625 patent, I disagree with Dr. Trout's opinion that claim 1 of the '625 patent is not anticipated by Boschelli 2001 because Boschelli 2001 does not disclose a pharmaceutical composition comprising bosutinib. Trout Report, ¶ 66.

55. *First*, I disagree that Boschelli 2001 does not disclose the use of pharmaceutical grade materials in the formulations administered to mice in the disclosed xenograft studies. As Wolff 2003 discloses, "[the use of non-pharmaceutical-grade materials] should be based on (1) scientific necessity, (2) nonavailability of an acceptable veterinary or human pharmaceutical-grade compound, and (3) specific review and approval by the IACUC." Wolff 2003 at 34. Wolff 2003 further notes that the use of non-pharmaceutical grade compounds could lead to the

inadvertent introduction of variables complicating the research. *Id*. In fact, in my own experience in the pharmaceutical industry, animal studies were frequently conducted with pharmaceutical grade materials and included active ingredients with high levels of purity. This was necessary because we could not risk our preclinical data being skewed by impurities in the materials we used. Thus, a POSA would have expected Boschelli 2001, which discloses that it is authored by Wyeth scientists, to have used pharmaceutical-grade materials and purity of active ingredient in the disclosed experiments.[4]

56. *Second*, I disagree with Dr. Trout that claim 1 of the '625 patent requires a composition comprising FDA-mandated purity of materials. Nowhere does the '625 patent suggest that "pharmaceutical composition" requires the components to meet particular purity levels. Nor does it disclose the use of any materials meeting USP-NF standards, including the use of any pharmaceutical-grade ingredients in the examples. In fact, claim 1 of the '625 patent does not even require that the composition be administered to humans.[5]

57. Instead, the '625 patent specification discloses generally that "[t]he compounds of the invention may be formulated with conventional excipients, such as a filler, a disintegrating agent, a binder, a lubricant, a flavoring agent, a color additive, or a carrier." '625 patent, col. 4:5-8. Moreover, the '625 patent discloses that "[w]hen provided orally or topically, such compounds would be provided to a subject by delivery in different carriers. Typically, such

[4] To the extent Dr. Trout asserts that a POSA would not have understood Boschelli 2001 to disclose the use of pharmaceutical-grade materials because that was not expressly specified, I note that the '625 patent also does not specify pharmaceutical-grade materials.

[5] During claim construction, Plaintiffs proposed that the term "pharmaceutical composition" be construed to mean ""a composition *suitable for administration to a human* containing the specified compound and one or more excipients." *See, e.g.*, D.I. 75 at 12. However, the Court rejected Plaintiffs' proposal that "pharmaceutical composition" require human administration. D.I. 98 at 1.

carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, or glycols." *Id.* at 4:17-21 (disclosing that typically, pharmaceutically acceptable carriers include sugars, such as dextrose). The '625 patent also expressly states that bosutinib can be delivered with a detergent such as Tween 80. *Id.* at 4:27-50. Based on this disclosure, a POSA would have understood that a "pharmaceutical composition" of bosutinib according to claim 1 of the '625 patent need only be a composition of bosutinib with a carrier and/or excipient such as the disclosed materials, such that the composition could be administered to an animal without killing the animal or causing severe morbidity.

58. As I explained in my Opening Report, Boschelli 2001 discloses and enables a composition comprising approximately 3 mg bosutinib, 10 mg Tween 80, and 25 mg dextrose/water in a 0.5 mL aqueous vehicle. These inactive ingredients are acceptable for use as a pharmaceutical composition in both animals and humans. *See, e.g.,* '625 patent at 4:17-21 (disclosing that sugars, such as dextrose, are suitable as a carrier) and 4:27-50 (disclosing Tween 80 as a detergent suitable for delivery of the present invention). Lindsley Opening Report, ¶ 162.

59. Because claim 1 of the '625 patent does not require a pharmaceutical composition to meet USP-NF standards to satisfy the scope of claim 1, and because a POSA would have nevertheless understood that Boschelli 2001 disclosed compositions comprising pharmaceutical-grade components, I disagree with Dr. Trout's opinion that Boschelli 2001 does not anticipate claim 1 of the '625 patent.

VI. THE ASSERTED CLAIMS ARE INVALID AS OBVIOUS OVER THE PRIOR ART

A. Obviousness As Of November 6, 2002

1. Claim 7 of the '148 patent is obvious over Donato 2003 in view of Boschelli 2001

60. Dr. Murcko and Dr. Shah offer the opinions that claim 7 of the '148 patent is not obvious over Donato 2003 in view of Boschelli 2001. I disagree with Dr. Murcko and Dr. Shah for at least the following reasons.

61. *First*, a POSA would have understood that Donato 2003 disclosed a method of treating CML with a therapeutically effective amount of a Src inhibitor. Dr. Murcko and Dr. Shah opine that Donato 2003's definition of a Src inhibitor actually describes BCR-ABL inhibitors (*see, e.g.,* Murcko Report, ¶ 114; Shah Report, ¶ 219), but both Dr. Murcko and Dr. Shah are referring to one particular phrase that has a very specific definition within Donato 2003, not Donato 2003's broader disclosure. That is to say, Dr. Murcko and Dr. Shah focus on the phrase "compounds inhibiting the c-Src protein tyrosine kinase activity" (*see id*.) which is defined in Donato 2003 as "such compounds having an IC_{50} in the range of 1 to 3000 nM, preferably in the range of 1 to 500 nM, in the proliferation test using BCR-ABL transfected 320 cells described hereinafter." Donato 2003 at 4. However, as I explained my Opening Report (Lindsley Opening Report, ¶ 175) and further below, Donato 2003 more broadly discloses "a method of treating a warm-blooded animal having leukaemia, in particular comprising administering to the animal at least one compound inhibiting the activity of a member of the Src kinase family… in a quantity which is therapeutically effective against leukaemia …" Donato 2003 at 3. Therefore, a POSA would have understood that Donato 2003 discloses the successful treatment of CML with a Src inhibitor.

62. *Second*, because Donato 2003 taught that Src inhibitors could be used to treat CML, a POSA would have been motivated to combine Donato 2003 with a reference that disclosed a known Src inhibitor, such as Boschelli 2001, and would have reasonably expected that using a Src inhibitor according to the method taught by Donato 2003 would effectively treat CML.

63. *Third*, a POSA would have further reasonably expected that using a Src inhibitor as disclosed by Boschelli 2001 according to the method taught by Donato 2003 would have successfully treated or inhibited the proliferation of CML because a POSA would have expected a given Src inhibitor to also inhibit BCR-ABL (Lindsley Opening Report, ¶¶ 166-168), and a POSA would have expected an Abl inhibitor to be able to successfully treat CML (*id*. at ¶¶ 166, 169-170).

64. In particular, a POSA would have known any given kinase inhibitor would have likely also inhibited another tyrosine kinase. Indeed, as Dr. Murcko points out, the cross-inhibition was so expected and common that skilled artisans had analyzed the inhibitory activity of a multitude of different compounds, including the ability of different compounds to inhibit both Abl and Src. *See, e.g.*, Murcko Report, ¶ 100 (citing Zimmerman et al., Ex. CC to Murcko Report). Table 2 of the Zimmerman reference, copied and pasted into the Murcko Report, discloses that while not all compounds that are strong Abl inhibitors are also strong Src inhibitors, all compounds that are strong Src inhibitors are, in fact, strong Abl inhibitors. *Id*. at Table 2 (disclosing that all compounds that inhibit c-Src with an IC_{50} of less than 20 μM are also v-Abl inhibitors). Thus, as Dr. Murcko's cited evidence suggests and supports, a POSA would have expected a known Src family kinase inhibitor to also be an inhibitor of Abl kinase.

65. In fact, the prior art disclosed that known Src inhibitors also turned out to be Abl inhibitors. *See, e.g.*, Dorsey 2000 (disclosing that PD180970, originally identified as a Src inhibitor, is also a strong BCR-ABL inhibitor); Tatton 2003 (disclosing that PP1, a Src inhibitor, is also an inhibitor of BCR-ABL); Warmuth 2002 (reporting that PP1 and CGP76030, originally identified as Src inhibitors, also inhibit Abl); Wisniewski 2002 (reporting that known Src inhibitor PD173955 was also an Abl inhibitor).

66. I further disagree with Dr. Murcko that a POSA would have required demonstrated inhibition activity in a BCR-ABL kinase assay or anti-proliferative activity in a CML-based cellular assay in order to have a reasonable expectation of success. Murcko Report, Section VIII.A. The prior art at the time disclosed that Src inhibitors could treat CML. Lindsley Opening Report, ¶¶ 167-168. Thus, a POSA reading the prior art, including Donato 2003, would have understood that agents other than an Abl inhibitor could be used to treat CML, and a POSA would not have required that a new drug candidate demonstrate activity specifically in a BCR-ABL kinase assay to reasonably expect that it could inhibit CML. In addition, because Donato 2003 expressly disclosed that Src inhibitors could successfully treat CML, a POSA would not have required demonstrated anti-proliferative activity in a CML-based cellular assay in order to reasonably expect that Src inhibitors could successfully treat CML.

67. I further disagree with Dr. Murcko and Dr. Shah that Warmuth 1997 suggests that a POSA would not have reasonably expected Src family kinase inhibitors to treat CML. Murcko Report, ¶ 111; Shah Report, ¶ 226. On the contrary, as Dr. Murcko and Dr. Shah observe, Warmuth 1997 acknowledged that BCR-ABL activates Src kinases; the only question was "to elucidate the *precise* mechanism of activation of Src kinases by BCR-ABL and the pathophysiological *role* of this interaction in Philadelphia chromosome positive leukemias"

(emphasis added). *Id*.; *see also* Warmuth 1997 at 33269. In other words, Warmuth 1997 did not question *whether* BCR-ABL activates Src kinases or *whether* this interaction played a role in Ph+ leukemias; the question, instead, was *how* BCR-ABL activated Src kinases, and *which* role that activation played in CML. These *how* and *which* questions would not have impacted a POSA's reasonable expectation that Src family kinase inhibitors could successfully treat CML.

68. I also disagree with Dr. Murcko's and Dr. Shah's opinion that Dorsey 2000 suggests that a POSA would not have had a reasonable expectation that Src family kinase inhibitors could successfully treat CML. Murcko Report, ¶ 112; Shah Report, ¶¶ 228, 241. Instead, Dorsey 2000 would have provided further support for a POSA to reasonably expect Src family kinase inhibitors could treat CML, because Dorsey 2000 discloses that the Src tyrosine kinase inhibitors PP1 and PP2 led to a significant decrease in K562 cell viability. *See, e.g.*, Dorsey 2000 at Figure 3D (disclosing that PP1 and PP2 treatment led to a decrease in cell viability of approximately 40% and 60%, respectively).

69. I also disagree with Dr. Murcko and Dr. Shah that Nimmanapalli 2002 suggests that a POSA would not have expected Src family kinase inhibitors to treat CML. Murcko Report, ¶ 113; Shah Report, ¶ 229. The statement in Nimmanapalli 2002 cited by Dr. Murcko and Dr. Shah only states that it is unclear whether PD1809070, a dual Abl and Src inhibitor, would be active in Gleevec-resistant cells, not whether PD1809070 is able to inhibit CML proliferation generally.

70. I also disagree with Dr. Murcko and Dr. Shah that Donato 2003 does not teach that administration of any compound that specifically inhibits Src family kinases would be effective for treating CML. Murcko Report, ¶ 114; Shah Report, ¶ 230. On the contrary, Donato 2003 discloses that Src family kinase inhibitors would be useful for the treatment of CML,

regardless of whether those compounds had also been shown to have anti-proliferative activity against $32D_{BCR\text{-}ABL}$ cells.

71. For example, as I explained in my Opening Report, Donato 2003 discloses that "the present invention relates to a method of ***treating a warm-blooded animal having leukaemia***, in particular comprising administering to the animal at least one compound inhibiting the activity of a member of the Src kinase family… in a quantity which is ***therapeutically effective against leukaemia***…" (emphasis added). Lindsley Opening Report, ¶ 175; *see also* Donato 2003 at 2.[6] While Donato 2003 does reference "compounds inhibiting the c-Src protein tyrosine kinase activity" (*see, e.g.*, Donato 2003 at 4), this term is separate from the broader term "compound inhibiting the activity of a member of the Src kinase family" or the term "compound decreasing the c-Src activity" (*see, e.g.*, Donato 2003 at 7), which is independently defined. In fact, as Dr. Shah notes, Donato 2003's definition of "compounds inhibiting the c-Src protein tyrosine kinase activity" "departed from the customary meaning of SRC kinase inhibitor as used in the art (inhibiting SRC kinase)." Shah Report, ¶ 230. A POSA would have therefore understood that Donato 2003 was providing a very particular definition limited to a very specific phrase, and would not have considered this particular definition to apply to other, separate and broader phrases such as "compound inhibiting the activity of a member of the Src kinase family," which Donato 2003 itself explains can include src, yes, hck, fyn, lyn, lck, blk, fgr or Yrk. Donato 2003 at 3.

72. Indeed, claim 6 of Donato 2003, for example, is directed to the broader term "one compound inhibiting the activity of a member of the Src kinase family, the Btk kinase family, the

[6] Page numbers in Donato 2003 reference document page numbers, not internal page numbers.

Tec kinase family or a Raf kinase inhibitor," and is not specifically limited to a "compounds inhibiting the c-Src protein tyrosine kinase activity" as it is particularly defined in Donato 2003.

73. Based on the disclosures of Donato 2003 relating to a "compound inhibiting the activity of a member of the Src kinase family," a POSA would have understood that Donato 2003 taught and suggested that a compound that could inhibit a Src family kinase would be effective to treat CML, regardless of whether that compound had been shown to have anti-proliferative activity against $32D_{BCR\text{-}ABL}$ cells.

74. In addition, as I explained in my Opening Report and above, a POSA would have been motivated to pursue Src kinase inhibitors to treat CML because Src kinase inhibitors were known to also be likely Abl inhibitors, and Abl inhibitors had been shown to effectively treat CML.

75. To the degree that Dr. Shah suggests that a POSA would have required clinical evidence in order to have a reasonable expectation that bosutinib could be effective to treat CML (*see, e.g.,* Shah Report ¶¶ 222, 235), I disagree.[7] It is my understanding that a claimed invention can be obvious if a POSA only had a *reasonable* expectation of success, not that the POSA knew with certainty that a claimed invention would work.

76. For all the reasons stated above, I disagree with Dr. Murcko and Dr. Shah that claim 7 of the '148 patent is not rendered obvious over Donato 2003 in view of Boschelli 2001.

2. Claim 1 of the '625 patent

77. As I explained above, claim 1 of the '625 is indefinite and therefore cannot be construed. However, to the extent "CML inhibiting amount" can be construed as

[7] To the extent that clinical evidence is necessary for a POSA to know that administration of bosutinib would be effective to treat CML, the asserted claims lack sufficient written description support and enablement, because neither the '148 patent nor the '625 patent discloses clinical evidence.

"therapeutically effective amount," and assuming a POSA would have understood the scope of claim 1 of the '625 patent, then for the same reasons I disagree with Dr. Murcko and Dr. Shah regarding the obviousness of claim 7 of the '148 patent, I disagree regarding the obviousness of claim 1 of the '625 patent.

B. Obviousness As Of November 6, 2003

78. I note that neither Dr. Murcko nor Dr. Shah has rebutted my opinions regarding obviousness of the asserted claims on the basis of prior art references available between November 6, 2002 and November 6, 2003.

79. Instead, Dr. Shah states Plaintiffs' counsel informed him that such references do not qualify as prior art because Plaintiffs have asserted an earlier invention date. As I explained in my Opening Report, according to industry practice, the named inventors were not diligent in reducing the claimed inventions to practice. Lindsley Opening Report, Section VII.D; *see also id.* at ¶¶ 8-9. Because Plaintiffs' Expert Reports do not address whether the named inventors were diligent, counsel for Defendants has informed me that Plaintiffs cannot now introduce new expert opinion regarding diligence. However, to the extent Plaintiffs' experts introduce new opinions at a later date, I reserve the right to respond to those opinions.

80. Dr. Shah also states that Plaintiffs' counsel informed him that Boschelli 2002 and Golas 2003 do not qualify as prior art because they are the work of the named inventors of the '148 and '625 patents. However, the CML mouse xenograft work that I rely on to analyze obviousness was not the work of the named inventors. *See, e.g.*, Lindsley Opening Report at ¶ 189 (citing Boschelli 2002's *in vivo* test results showing that oral administration of bosutinib to mice bearing K562 CML xenografts led to tumor regression); ¶¶ 195-196 (citing *in vivo* mouse studies using K562 CML xenografts). Instead, the mouse xenograft data was obtained from the work of Judy Lucas or those under her direction, and Judy Lucas is not a named inventor. *See*,

e.g., Golas Dep. Tr. 47:21-48:3 (testifying that Judy Lucas's group handled *in vivo* xenograft studies), 69:2-8 (testifying that the data from Table 3 of the '148 patent would have been obtained from Judy Lucas's group); *see also* Lucas Dep. Tr. 40:12-15 (testifying that Judy Lucas conducted the testing to obtain the data for Table 3 of the '148 patent), 150:6-152:2 (testifying that Table 3 of the '148 patent contains the same data as Table 1 of Golas 2003). I understand that Boschelli 2002 and Golas 2003 can still be relied on as prior art because the data I am relying on was not the work of the named inventors of the '148 and '625 patents.

81. Therefore, my opinions that claim 7 of the '148 patent and claim 1 of the '625 patent are obvious over Donato 2003 and Boschelli 2001 further in view of Boschelli 2002, as well as obvious over Golas 2003, remain the same and unrebutted.

C. No Secondary Considerations Support The Nonobviousness Of The Asserted Claims

1. The Asserted Claims Did Not Meet A Long-Felt But Unresolved Need

82. It is my understanding that Plaintiffs bear the burden of demonstrating that there are secondary considerations indicative of nonobviousness. I further understand that, for long-felt need, this burden includes (a) identifying the long-felt need, (b) showing that the asserted claims are commensurate in scope with the long-felt need, and (c) demonstrating that the claimed invention met such need. Dr. Shah did not do so here.

83. First, Dr. Shah did not identify the long-felt need that the asserted claims supposedly met. Therefore, it is unclear to me whether Plaintiffs are asserting that the claims met a long-felt need for treating CML generally, Gleevec-resistant CML only, or something else entirely.

84. Assuming that that Plaintiffs are asserting that the claims met a long-felt need to treat Gleevec-resistant CML[8], the claims are not commensurate in scope with the purported long-felt need. The asserted claims are directed to the treatment of CML generally and compositions comprising a CML inhibiting amount of bosutinib; they are not limited to treating Gleevec-resistant CML patients or compositions for the treatment of such patients. Because treating Gleevec-resistant patients and compositions for treating Gleevec-resistant patients is only a small subset of the claim scope, satisfaction of this purported need – even if long-felt – cannot support the nonobviousness of the asserted claims.

85. Regardless, as I explained in my Opening Report, the asserted claims did not meet a long-felt need at the time of the patent filing date, because not enough time elapsed between the approval of Gleevec and the asserted patents' filing date to qualify as a "long-felt" need for a treatment for Gleevec-resistant CML.

86. Assuming that the need to treat Gleevec-resistant CML was recognized by or shortly before FDA approval of Gleevec in 2001, only about 2.5 years elapsed between Gleevec's approval in May 2001 and the asserted patents' filing date in November 6, 2003. Drug discovery takes, on average, 12 years from identification of a potential drug candidate to clinical trial validation of that drug's ability to successfully treat the target indication, assuming every study succeeds along the way and as hoped. *See*, *e.g.*, Norman 2016 at 170. For example, it took approximately 12 years from the identification of bosutinib to FDA approval in 2012. Thus, for there to be any "long-felt" need, there must have been enough time for drug developers to attempt to try and fail to resolve the problem after that need was first recognized. Therefore,

[8] To the extent Plaintiffs are alleging that the claims satisfied a long-felt need for a CML treatment, generally, I disagree. This need was met by Gleevec, which was approved before the filing of the applications leading to the '148 and '625 patents.

at the very least, there could not have been a "long-felt" need until at least 10 years after the need was first recognized. Here, because the asserted patents were filed only 2.5 years after a need to treat Gleevec-resistant CML was first recognized, there was no long-felt need to treat Gleevec-resistant CML.

87. Moreover, to the extent that long-felt need is considered a secondary consideration of nonobviousness because a long time had elapsed before a solution was reached, I note that as soon as Gleevec was approved, multiple parties were developing Abl inhibitors for the treatment of CML, such as nilotinib and dasatinib. For example, nilotinib, the active ingredient of the CML drug TASIGNA®, is another Abl inhibitor that treats Gleevec-resistant or Gleevec-intolerant CML patients. Enrollment of CML patients in a clinical trial for nilotinib began as early as May 2004[9], indicating that successful preclinical development of nilotinib had been underway beforehand, and TASIGNA was approved by the FDA for the treatment of CML in 2007, five years earlier than BOSULIF®.

88. Likewise, dasatinib, also known as BMS-354825, is a dual Src and Abl inhibitor and the active ingredient of the CML drug SPRYCEL®, and had been patented at least as of July 2003 (*see* U.S. Pat. No. 6,596,746). A patent application for the use of dasatinib to treat Gleevec-resistant CML was filed as early as March 2003 (*see* U.S. Pat. No. 7,125,875). Enrollment of CML patients in a clinical trial for dasatinib began as early as November 2003[10],

[9] This study, "A Phase IA/II Multicenter, Dose-escalation Study of Oral AMN107 on a Continuous Daily Dosing Schedule in Adult Patients With Imatinib-resistant/Intolerant CML in Chronic or Accelerated Phase or Blast Crisis, Relapsed/Refractory Ph+ ALL, and Other Hematologic Malignancies," was registered as clinical trial NCT00109707, *available at* https://clinicaltrials.gov/ct2/show/NCT00109707.

[10] This study, "A Phase I Dose-Escalation Study To Determine The Safety, Pharmacokinetics, And Pharmacodynamics Of BMS-354825 In The Treatment Of Patients With Chronic Phase Chronic Myelogenous Leukemia Who Have Hematologic Resistance To Imatinib Mesylate

indicating that successful preclinical development of dasatinib had been underway beforehand, and SPRYCEL was approved by the FDA to treat CML in 2006—six years earlier than BOSULIF®.

89. The fact that these Abl inhibitors would not gain FDA approval for years after the filing date of the asserted patents is characteristic of the industry; as I explained above, it takes a long time before proof of concept to clinical trial validation of a given drug candidate. Therefore, the 2.5 years between Gleevec approval and the filing date of the asserted patents without FDA approval of additional CML drugs merely indicates that it takes a long time for pharmaceutical companies to develop and gain FDA approval for new drugs.

2. The Asserted Claims Did Not Demonstrate Unexpected Results

90. As for long-felt need, it is my understanding that it is Plaintiffs' burden to demonstrate unexpected results, including (a) a comparison of the purported unexpected results with the closest prior art; (b) a demonstration of nexus between the purported unexpected results and the asserted claims; (c) a showing that the purported unexpected results are commensurate with the asserted claims; and (d) an explanation of how such results are truly unexpected. Dr. Shah has not demonstrated any of these things.

91. *First*, Dr. Shah has not identified the closest prior art, and there can be no unexpected results without a comparison with the closest prior art. In my opinion, the closest prior art is Donato 2003, which discloses that Src inhibitors (especially Src inhibitors that can also inhibit BCR-ABL) can be used to treat imatinib-resistant CML. *See, e.g.*, Donato 2003 at claim 13 (directed to "Method according to claim 6, 7, 9 or 11 wherein said leukaemia is resistant to monotherapy employing N-(544-(4-methyl-piperazino-methyl)-benzoylamido]-2-

(Gleevec)," was registered as clinical trial NCT00064233, *available at* https://clinicaltrials.gov/ct2/show/NCT00064233.

methylpheny1)-4-(3-pyridy1)-2-pyrimidine-amine as sole active agent." There is no reason to believe that bosutinib's ability to inhibit imatinib-resistant BCR-ABL kinase or to treat imatinib-resistant patients would have been unexpected compared to Donato 2003. Quite the contrary, Donato 2003's disclosure that Src inhibitors can be used to treat imatinib-resistant CML would have suggested precisely those results to a POSA.

92. *Second*, to the extent Dr. Shah asserts that the unexpected results are bosutinib's ability to inhibit 16 of 18 imatinib-resistant forms of BCR-ABL kinase in *in vitro* studies with murine myeloid cell lines (Shah Report, ¶¶ 274-275), I note that there is no nexus between this result and the asserted claims. The asserted claims are not directed to the bosutinib compound itself. Plaintiffs argue that claim 7 of the 148 patent is directed to a method of treating human patients with CML with a therapeutically effective amount of bosutinib. The method of claim 7 does not treat murine myeloid cell lines grown *in vitro*. Thus, there is no nexus between Plaintiffs' asserted unexpected results and claim 7. For the same reason, there is no nexus between Plaintiffs' asserted unexpected results and claim 1 of the 625 patent—no pharmaceutical composition was administered to murine cell lines *in vitro*.

93. *Third*, to the extent Dr. Shah opines that unexpected results were demonstrated because bosutinib successfully treated imatinib-resistant and imatinib-intolerant patients in clinical trials, that result is not commensurate with the scope of the asserted claims, which are not limited to imatinib-resistant or imatinib-intolerant patients. Instead, the asserted claims are directed to CML generally.

94. *Fourth*, it would not have been unexpected that bosutinib could be used to treat imatinib-resistant or intolerant CML because bosutinib is a structurally unrelated compound with a not-identical mechanism of action and was known to be a Src inhibitor, so would be expected

to inhibit CML even if BCR-ABL kinase mutation made it unresponsive to Abl inhibition, either by imatinib, bosutinib, or another Abl inhibitor.

95. For the reasons stated in my Opening Report and above, Dr. Shah and Plaintiffs have not provided evidence showing unexpected results indicative of nonobviousness of the asserted claims.

VII. SUPPLEMENTAL OPINIONS

96. In the event that Plaintiffs submit any supplemental report in response to this reply expert report, I reserve the right to respond to any issues raised by such a response.

97. If called to testify at trial, my testimony may include an explanation of the scientific principles that underlie the opinions expressed in this report.

98. I have based my opinions and analysis on documents and information available to me at the time I signed this report. If and when any new evidence or arguments arise, I reserve the right to supplement or modify my opinions to reflect those evidence or arguments.

99. I reserve the right to make and use demonstratives to help explain my opinions.

VIII. COMPENSATION

100. I am being compensated at the rate of $400/hour. My compensation is not dependent on the opinions I express or the outcome of this lawsuit. I do not have any financial interest in any party in this litigation.

IX. PRIOR TESTIMONY

101. I have not testified as an expert at trial or deposition in the last four years.

Dated: June 27, 2019

Craig W. Lindsley